AUSTRALIA

Patents Act 1990

IN THE MATTER OF Australian Patent Application Serial No. 696764 by Human Genome Sciences, Inc.

-and-

IN THE MATTER OF Opposition thereto by Ludwig Institute for Cancer Research

STATUTORY DECLARATION

I, Francis John Ballard, of 21 Willowbridge Grove, Burnside, South Australia, 5066, do solemnly and sincerely declare as follows:

I. INTRODUCTION

1.1 Ludwig Institute for Cancer Research ("Ludwig Institute") has asked for my services as a scientific expert in connection with Ludwig Institute's opposition to the issuance of an Australian patent to Human Genome Sciences, Inc., ("HGS") based on HGS's Australian Patent Application No. 696764. The patent application relates generally to an isolated polynucleotide and protein for an alleged novel vascular endothelial growth factor called "Vascular Endothelial Growth Factor 2" ("VEGF2").

1.2 The first evidence that I provided in the opposition proceeding was a declaration from February 2000 (hereinafter referred to as "OJB1" (Opponent, John Ballard, 1st Declaration)). My first declaration included a brief resume of my scientific experience, and explained that I had reviewed and agreed with a detailed declaration executed by Peter Rogers (hereinafter "OPR1"), to which I will refer again in this declaration.

1.3 In answer to Ludwig Institute's initial evidentiary submission, HGS filed declarations from six scientists, John Stanley Mattick (hereinafter "AJM1"(Applicant,

John Martick, 1st Declaration)), Jennifer Ruth Gamble ("AJG1"), Nicholas Kim Hayward ("ANH1"), Thomas Rapoport ("ATR1"), Stuart Aaronson ("ASA1"), and Susan Power ("ASP1").

1.4 In reply to HGS's declarations, Ludwig filed second declarations from Dr. Alitalo (OKA2), Dr. Rogers (OPR2) and me (OJB2). HGS then obtained special permission to file still further evidence, in the form of supplemental declarations by Dr. Power (ASP2), Dr. Hayward (ANH2), and Dr. Aaronson (ASA2). Ludwig Institute asked me to review these most recent declarations filed by HGS and evaluate them in the context of all of the documents that have been filed in this proceeding.

1.5 I hereby reaffirm my understanding that I have an overriding duty to the Patent Office (and to any Australian Federal Court that should review the Patent Office decision) to provide objective scientific analysis that I believe to be truthful. I hereby affirm that, to the best of my knowledge and belief, factual statements herein are true and opinion statements herein represent my objective scientific opinion and analysis.

1.6 Unless I specifically state otherwise below, I affirm the facts and opinions expressed in my prior declarations (i.e., in OJB1/OPR1 and OJB2). Nothing in HGS's evidence-in-answer causes me to change the opinions embodied in my earlier declarations. (In fact, it is worth observing at the outset that HGS did not specifically dispute my second declaration in the further evidence that they submitted.)

II. THE CRITICISMS OF DR. ALITALO'S SECOND DECLARATION ARE MERITLESS.

2.1 Notwithstanding the further evidence filed by HGS, in my opinion Dr. Alitalo's experiments remain the only experimental evidence in this proceeding directed to the question of whether VEGF2, as taught in the opposed application, is expressed and secreted by cells. As reported in his first two declarations, Dr. Alitalo has repeatedly found that expression and secretion does not occur. Drs. Aaronson and Hayward urged that these experiments were defective and the results should be ignored, but their criticisms are unfounded. Having analyzed Dr. Alitalo's reported experiments and results, and also all of the criticisms by Drs. Aaronson and Hayward, I continue to hold the opinion that the Alitalo experiments are the most relevant to the teachings of the opposed application and are

scientifically sound. I discuss some of the problems with the Aaronson-Hayward analysis in this section.

A. Failure to use a heterologous signal sequence.

2.2 The HGS declarants urge that it was a "flaw" for Dr. Alitalo not to report on the effect of attaching a heterologous signal sequence to the 350 amino acid VEGF2 sequence. (See, e.g., ANH2 at 1.5, 1.6, 1.10; ASA2 at 5, 6-10, 13-22.) The flaw is not in Dr. Alitalo's experiments, but rather, in HGS's reasoning. As extensively explained in Ludwig Institute's second evidentiary submission, the opposed application does not teach to perform the experiments urged by HGS. [OJB2 at 2.2, 3.19-3.24, 6.1-6.6,; OPR2 at 4.4-4.6, 4.12, 4.14-4.53.]

2.2.1. Dr. Alitalo's work shows that a scientist in 1994 attempting to express the VEGF2 sequence by following the teaching of the opposed application would have been unsuccessful, e.g., because as extensively explained in the Ludwig Institute's evidence and sometimes admitted by HGS declarants, the VEGF2 of the opposed application is incomplete. It is missing about 69 codons, including the codons for the signal sequence. That scientist would have then needed further experimentation to identify the source of the problem or problems and determining the solution or solutions to those problems. However, without the benefit of hindsight that we now enjoy, that scientist would not have known that the problem involved a missing signal peptide and other N-terminal sequences. The absence of a signal sequence would not, in my opinion, have been the only possibility nor would it have been the first possibility as to the source of the problem. In fact, I believe the scientist would have been comforted and guided by the express statements in the opposed application that the VEGF2 sequence as taught therein includes a signal sequence. It is interesting to observe that when HGS discovered the problem, and its solution, they considered the discovery to be significant enough to file a second series of patent applications on the 419 amino acid form of VEGF2. In this opposition proceeding, the HGS declarants are able to focus directly on the problem of a signal peptide, but only through the benefit of hindsight knowledge.

2.3 In my opinion, Dr. Alitalo's experiments were properly designed to test the opposed application's teachings, which are that full length VEGF2 comprises 350 amino acids, of which approximately the first 24 amino acids are a signal sequence. (See, e.g., opposed application at p. 4.) These were the specific teachings to the public about the subject

matter of the opposed application. The experiments urged by HGS are a form of revisionist history - an attempt to fix a defective patent application by hindsight knowledge of a protein learned from publications by Dr. Alitalo and others. [See, e.g., Documents D70-D73.] The question as I understand it is not whether a person in year 2001 or 2002, knowing what Dr. Alitalo and others have taught the public about the VEGF-C gene and protein, can apply that knowledge and succeed at expressing some portion of the VEGF2 sequence taught in HGS's 1994 patent application. The question is whether the teachings of the application were accurate and placed the public in possession of a complete and working invention. Dr. Alitalo tested the teachings of the opposed application and showed that the answer to this question is no.

B. Controls

2.4 I find nothing unreliable in Dr. Alitalo's experiments or conclusions. With almost any experimental summary, a scientist with general knowledge in a field can identify some parameters for which the experimenter did not report controls. At the same time, such a scientist is also familiar with which aspects of an experiment are routine or uncontroversial, and which are more critical to the variable being tested. In my opinion, none of the controls on which the HGS experts focus are serious experimental omissions or omissions that could reasonably be expected to affect the experimental conclusions of Dr. Alitalo, if indeed Dr. Alitalo omitted them at all.

2.5 Dr. Alitalo ran parallel experiments where the only meaningful variable which he manipulated was the identity of the insert in the expression vector. The VEGF-C "positive control" insert worked just fine, because it has a signal peptide, but the VEGF2 as taught in the opposed application failed, because it does not. The use of positive (VEGF-C) and negative (mock transfection/empty vector) controls in Dr. Alitalo's experiments were the meaningful controls that a scientist with general knowledge in the field of study would have looked for.

C. Transfection Efficiency, cell density, and cell growth conditions.

2.6 The HGS experts alleged that Dr. Alitalo's experiments failed to report details of the transfection efficiency of the plasmids used in the experiments, cell densities, or cell growth conditions. (ANH2 at 1.5; ASA2 at 23-24.) These criticisms are defective for several reasons.

2.7 First, in my opinion, the criticisms are an attempt to distract the Patent Office from important details by drawing excessive attention to insignificances. The issues of transfection efficiency, cell density, and cell growth conditions are not variables that I would have expected to read in an experiment report such as Dr. Alitalo's OKA2 declaration, because of the high degree of experience in the field with these technologies. For example, they are not details which I would expect a scientist to include in a manuscript submitted to a journal for peer review and publication because the editors would not question these details.[1]

2.8 Second, when judging the controls that Dr. Alitalo reported in OKA2, one must also remember the context in which that document was created. Dr. Alitalo had already performed perfectly adequate experiments based on the opposed application and reported his results in OKA1. The experiments reported in OKA2 were performed to address specific criticisms of OKA1 raised by HGS in their first series of declarations. (See OKA2 at 1.2, 3.1, and 3.11-3.12, for example.) Dr. Alitalo reported those details which showed that he had considered and controlled for the variables that HGS's declarants allegedly had found problematic with his first declaration. Given that Dr. Alitalo's instructions for his reply declaration were "to design and perform further protein expression studies that would address concerns raised by AJG1, ANH1, and ASA1 directed towards my first declaration," it should not be surprising to any reader that he did not report on the details about which Drs. Hayward and Aaronson are now complaining. Neither Dr. Hayward nor Dr. Aaronson raised these issues in their first declarations, necessitating no reply by Dr. Alitalo.

2.9 Third, and most importantly, these criticisms are rendered moot by the parallel design of Dr. Alitalo's experiments. It is apparent to me that Dr. Alitalo used the same procedures for the VEGF2 clone and the positive (VEGF-C insert) and negative (no insert) controls. Had Dr. Alitalo ran his transfections or cell cultures defectively, then he would not have seen a positive result with the VEGF-C expression. The visible VEGF-C expression shows that there were no transfection problems, cell density issues, or cell growth

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[1] For example, , in a paper co-authored by Dr. Aaronson (Breuninger *et al., Cancer Res. 55*:5342-5347 (1995)) that looks at the expression of a multidrug resistance protein in NIH/3T3 cells, there is no description of any transfection efficiency experiments. In another paper co-authored by Aaronson (Beeler *et al., Mol. Cell. Biol. 14*:982-988 (1994)) that looks at the expression of a tyrosine kinase in bacterial cells, the expression analysis was carried out a single time-point (2 hours), a single temperature (37°C), and at a single concentration of IPTG (100 μM). Dr. Aaronson did not seem to be concerned that readers would consider his data meaningless in these papers, notwithstanding the "missing" controls.

condition problems with his experimental procedures, and that the failure to observe VEGF2 expression was due to properties of the VEGF2 insert. Stated differently, because the experimental procedures were the same for VEGF2, VEGF-C, and the empty vector, the differences in results are due wholly to the insert employed. It is apparent from the experimental design that, if transfection efficiency or cell growth were defective for VEGF2, the defect can only be attributed to properties of VEGF2, because the VEGF-C clone that was transfected and grown the same way expressed without problems. The design of the Alitalo experiments controlled for all meaningful variables except the variable being tested, i.e., whether there was a defect in the VEGF2 clone as taught in the application that prevented it from being expressed and secreted. I agree with Dr. Alitalo that the absence of a functional signal peptide, admitted in HGS's first series of declarations [see, e.g., AJM1 at 4.13; ANH1 at 3.13; AJG1 at 6.4; ATR1 at 8.], is a probable explanation. However, whatever the scientific explanation, it is attributable to defects in VEGF2 taught in the opposed application, and not defects in Dr. Alitalo's experimental design.

D. Time Course of Experiments

2.10 HGS's declarants criticized that Dr. Alitalo's experimental protocol "does not allow for detection of VEGF2 protein expression over various time points. Rather, protein levels are assessed fifty hours post-transfection (forty-eight hours and overnight metabolic labeling)." (ANH2 at 1.5; see also ASA2 at 25.)

2.11 The criticism of using 50 hour, or 48 hour, or overnight time points would appear to be rendered moot by Dr. Power's declaration, because she chose similar time points. Dr. Power states that protein expression was visible at both 24 and 48 hours in her experiments. [See ASP2 at 19 ("The transfected cells were cultured for 24 or 48 hours to allow for expression of the gene products encoded by each vector."); 22; and 31 ("The secreted protein was visible at 24 hours and 48 hours after transfection.").] In fact, it appears to me that she observed greater expression at 48 hours than 24 hours, although she may have adjusted her sample size during gel loading to compensate for this effect.

2.12 The criticism is further rendered moot by Dr. Alitalo's use of a positive control (the VEGF-C plasmid). Had Dr. Alitalo ran his experiments for too short a time, he would not have seen a positive result (VEGF-C expression) with the VEGF-C clone. The visible VEGF-C expression shows that the failure to observe VEGF2 expression was not due

to inappropriate time points, but instead to problems with VEGF2 as taught in the opposed application.

2.13 Dr. Aaronson seems to be urging that comparative analysis using the incomplete 350 amino acid VEGF2 form taught in the opposed application or a full length 419 form "is meaningless because disparate conditions and parameters will affect the expression, secretion and processing profiles of 350 amino acid VEGF-2 and 419 amino acid VEGF-2." This speculation is entirely unsupported by any reported experiments by Dr. Aaronson. Dr. Aaronson's opinion also appears to be belied by Dr. Power's work, and contradicts Dr. Hayward's professed opinion that by March 1994 he was aware that any given host cell would possess the proteolytic enzymes and cellular machinery to naturally process a protein such as VEGF2 to its mature form. (See ANH2 at 1.7.)

2.14 One other fact is clear. Even if Dr. Aaronson's opinion on this issue had merit, then one would still reach the conclusion that the defect lies not with Dr. Alitalo, but with the opposed application. After all, if disparate conditions are required, the opposed application fails to teach to the Australian public the special conditions that are required to express the 350 amino acid VEGF2 as taught in the opposed application.

E. Dr. Aaronson mischaracterized the Alitalo II declaration.

2.15 Dr. Aaronson declared as follows: "Dr. Alitalo also speculates that the VEGF2 as taught in the HGS patent specification is not secreted, but rather is rapidly degraded in cells. (see Alitalo Declaration II ¶ 3.9)." (See ASA2 at 25.) The entirety of Dr. Alitalo's analysis in that paragraph was that the protein was likely rapidly degraded *if the truncated protein is synthesized at all.* Dr. Alitalo's complete explanation for failure to observe secreted protein was omitted from Dr. Aaronson's analysis.

III. THE HGS DECLARATIONS SUPPORT LUDWIG INSTITUTE'S POSITION THAT THE HGS APPLICATION FAILS TO DESCRIBE A WORKING INVENTION

3.1 The HGS declarants have urged that the Patent Office adopt very stringent criteria in this opposition proceeding for evaluation of scientific data. As set forth in this section, if HGS's own unreasonable standards were applied to the application, one would have to conclude that the opposed application itself is wholly defective.

3.2 For example, Dr. Hayward and Dr. Aaronson have urged that no meaningful conclusions can be drawn from reports of scientific experiments for which data on all positive and negative controls are not provided to the reader for evaluation. (See, e.g., ANH2 at 1.5; ASA2 at 5, 12, and 23.) However, their remarks were directed to the experiments reported in the Alitalo declarations (concerning VEGF2 expression). Ludwig Institute asked me to review the Hayward and Aaronson declarations and then evaluate the science reported *in the opposed application* using a similar standard.

3.3 The first experiments reported in the opposed application are found in Example 1, a Northern blot analysis. Applying the same logic used by HGS' declarants, Drs. Hayward and Aaronson, one would require a positive control to validate the hybridization procedure. For example, one would expect parallel hybridization data for a known gene that had been well studied by 1994 and whose expression in various tissues and cancer cell lines has previously been established. This results for the positive control could be compared with the results for the VEGF-C and would ensure that the parameters for the Northern blot analysis will properly detect gene-specific hybridization. Likewise, one would require parallel analysis of a negative control, such as analysis of a gene whose expression has previously been determined to be silent (or a foreign gene that is absent) in the various tissues and cancer cell lines tested. Such a control is important to rule out false positives as a result of experimental design imperfections. One would require a positive control lane that was intentionally loaded with VEGF2 RNA and a negative control that was intentionally loaded with a sample known to be free of any VEGF2 RNA. No such controls are described. Also, Example 1 fails to indicate that any care was taken to use similar amounts of cells, or to use cells at similar stages of growth. According to the standards set by the HGS declarants, no meaningful conclusions can be drawn from Example 1. In fact, as explained previously, Northern hybridization studies by other groups have proven that the results reported in Example 1 are highly suspect, (See OPR1 at 4.13-4.13.1.)

3.4 Example 2 of the opposed application also lacks any reported positive and negative controls, such as a <u>known</u> gene that was successfully expressed in the <u>in vitro</u> reticulocyte lysate system and a promoterless gene that was not. There were no reported controls to assure that the PCR products used for expression contained VEGF2 sequence (as opposed to impurities that may have been amplified). There were no reported controls or tests performed to assure that the translated products were VEGF2 proteins. According to the standards set by the HGS declarants, no meaningful conclusions can be drawn from Example

2, because of the lack of any reported controls. In fact, the results reported in Example 2 (a 38-40 kDa protein) are inconsistent with the results that Dr. Power reports in her declarations in "support" of HGS (a 30 kDa protein).

3.5 Another alleged criticism of Dr. Alitalo's work, raised by Dr. Hayward and Dr. Aaronson, was with regard to analysis of protein expression over a time course, rather than a single time point. However, no such time course is evident in Example 2 of the opposed patent in which protein expression is analyzed. Similarly, HGS failed to report timing considerations for the Northern blot analysis of Example 1 and the conditions and circumstances in which samples of human tissues and cancer cell lines were collected and prepared or cultured. Thus, applying the standards urged by HGS's experts for scrutiny of experimental design, I would apply Dr. Aaronson's own words to the examples of the opposed application:

> "As discussed above, in the absence of appropriate controls, comparative analysis is meaningless because disparate conditions and parameters will affect the expression, secretion, and processing profiles....In any comparative analysis, results are meaningless without assurance that unnecessary variables are eliminated. The failure to include basic experimental controls to ascertain that there would be no problems with the expression vectors, cells, ... precludes making any meaningful conclusions."
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> (ASA2, par. 26)

3.6 Everything else in the opposed application (besides Examples 1 and 2) appears to be pure speculation, unsupported by any reported experimentation whatsoever. For example, the speculation about a signal peptide and mature VEGF2 is based on no reported experimentation, and has been proven wrong by Dr. Alitalo. The application also contains no reported experiments regarding VEGF2 biological activity. Applying the Hayward-Aaronson standard, one must conclude that assertions unsupported by experimentation are even less reliable than assertions based on experiments which were performed, but which lacked a complete set of positive and negative controls. Thus, applying the HGS analytical standard, I would conclude that no meaningful conclusions can be drawn from the opposed application regarding any structural or functional properties of VEGF2.

3.7 The HGS declarations do not apply the same standards for scientific review to the opposed application of HGS that they apply to the Ludwig declarations. Paragraphs 10-11 of Dr. Aaronson's second declaration provides a succinct example of the

biased analysis that the HGS declarants are offering in this proceeding. Dr. Aaronson expresses the opinion that one familiar with the molecular biology of growth factors and "equipped with the HGS patent specification would recognize that the 350 amino acid polypeptide is a secreted growth factor . . . ." (ASA2 at 10.) Dr. Aaronson characterizes this as a "literal teaching of the HGS patent specification which describes VEGF-2 as a secreted growth factor . . . ." (ASA2 at 11.) Dr. Aaronson has apparently overlooked or excused the fact that the opposed application contains no controlled experiments directed to trying to express and secrete that protein or determine if it is a growth factor. According to the very standards that Dr. Aaronson articulated for evaluating the Alitalo experiments, the skilled molecular biologist *should* conclude that the opposed application "fail[s] to provide any meaningful information regarding the expression, processing and secretion of VEGF-2" or its use as a "growth factor" for any purpose. (Compare ASA2 at 5 and 12.)

IV. INCONSISTENCIES IN THE HGS DECLARATIONS.

4.1 It is apparent to me that there are a number of unexplained inconsistencies made by the HGS declarants. I have highlighted some examples below.

A. Dr. Power

4.2 As explained below in greater detail, Dr. Power says in her first declaration that she used the deposited VEGF2 clone cross-referenced in the opposed patent application. In her second declaration, she admits that this was false.

B. Dr. Hayward and Dr. Aaronson

4.3 Dr. Hayward and Aaronson both say that "VEGF-2" and "VEGF-C" are alternate names for the same protein (ANH2 at 1.4; ASA2 at 3.) However, substantial parts of their declarations strenuously assert that parallel experiments by Dr. Alitalo, which were identical in essentially every respect except for use of either a full length VEGF-C clone or the incomplete VEGF2 taught in the opposed application, are totally unsound and unreliable, e.g., because of the disparate nature of the clones used.

4.4 Dr. Aaronson urges that Dr. Power's work shows that the 350 amino acid and 419 amino acid forms of VEGF2 are similarly processed (ASA2 at 28), but urges that Dr. Alitalo's parallel experiments that were actually based on the teachings of the application are unreliable because disparate conditions and parameters will affect the

expression, secretion and processing profiles of 350 amino acid VEGF2 and 419 amino acid VEGF2. (ASA2 at 26.)

4.5 In his first declaration, Dr. Hayward openly admitted that the opposed application discloses only 350 amino acids of the VEGF2 sequence which has subsequently been determined to have 419 amino acids. He and others admit that "The missing amino acid sequences is now known to contain the signal sequence that directs secretion of VEGF-2 from the cell." (See, e.g., ANH1 at 3.13.) In his second declaration, Dr. Hayward says that proteolytic processing is determined by a protein's amino acid sequence and that he would have expected that the 350 amino acid VEGF2 "contained all the necessary information and signals required by a host cell to process the amino acid sequence to its mature form." (ANH2 at 1.7.)

4.6 In his first declaration, Dr. Hayward expressed the opinion that data in the first Alitalo declaration was unreliable because of inconsistencies in expression and/or processing by different cell lines used for VEGF2 expression studies. (See ANH1 at 5.5.) Now, in his second declaration, he asks the Patent Office to believe that "By March 1994 I was aware that any given host cell would possess the proteolytic enzymes and cellular machinery to naturally process a protein such as VEGF-2 to its mature form." (ANH2 at 1.7).

4.7 Drs. Hayward and Aaronson both profess to be evaluating the application as they would have in 1994. (See, e.g., ANH1 at 2.1 and ANH2 at 1.2; ASA2 at 4.) However, they consistently and impermissibly try to draw analogies between incomplete VEGF2 taught in the opposed application in 1994 with VEGF-C research conducted by Dr. Alitalo using a different molecule, and not reported by Dr. Alitalo in the public literature until 1996-1997.

4.8 These examples of inconsistencies when evaluating the subject matter of this proceeding confirms the lack of objectivity of the HGS declarants.

V. HGS'S FURTHER EVIDENCE CONFIRMS LUDWIG INSTITUTE'S EVIDENCE

A. Dr. Power Confirms That Her First Declaration Was Inaccurate (Response to ASP2 paragraphs 1-10.)

5.1 In his second declaration, Dr. Alitalo summarized some simple sequencing experiments which demonstrated that Dr. Susan Power's first declaration contained falsehoods/inaccuracies. In particular, Dr. Power had indicated in her first declaration that she performed some experiments with the HGS clone that had been deposited with the ATCC and that was cross-referenced in the opposed application (ATCC Clone 75698). Dr. Alitalo obtained ATCC Clone 75698 directly from the ATCC, sequenced it, and showed that Dr. Power could not possibly have obtained the results that she said that she obtained by using the deposited clone. (See OKA2 at 4.1-4.3 and 5.4.) Dr. Power admits that her first declaration described her experiments inaccurately (see ASP2 at 6, for example), and that Dr. Alitalo's analysis was accurate. (See, e.g., ASP2 at 9.) The use by Dr. Power of a different clone (ATCC Clone 97149) containing a longer VEGF2 sequence (419 amino acids) that was not taught in the opposed application, and was not actually referred to by HGS until its second generation of VEGF2 patent applications filed more than a year after the filing of the opposed application (see, e.g., ASP2 at 5-6) renders her conclusions irrelevant.

5.2 Dr. Power appears to me to be suggesting in her second declaration that the inaccuracies in her first declaration were a result of being under "significant time constraints". (See ASP2 at 6.) However, even if she were under time constraints, true candor and scientific process requires that she should have accurately described her shortcuts, not hidden them. This is not really further evidence, so much as an attempt to rehabilitate her "old" evidence.

5.3 In any event, if Dr. Power were already in possession of ATCC Clone 75698, as she seems to suggest in ASP2 at paragraph 5, she would not be saving herself any time by attempting to recreate that clone using another clone (ATCC 97149). There is no economy of time in attempting to recreate something that you already have in your possession.

5.4 However, Dr. Power's second declaration may serve to illuminate confusion that exists as to the precision or accuracy of the description of ATCC Clone 75698 to one who reads the patent application. HGS's own expert failed to recreate the clone successfully from the description in the application. In fact, HGS itself does not seem to

understand the nature of the clone because they continue to ask Dr. Power "to specifically use the VEGF-2 coding sequence contained in ATCC Deposit No. 75698 to generate the expression construct containing the 350 amino acid for of VEGF-2." (ASP2 at 8.) As first explained by Dr. Alitalo and now acknowledged by Dr. Power, the ATCC clone does not contain 350 amino acids. (OKA2 at 4.1-4.3 and 5.4; ASP2 at 9.) It is hard to imagine a clearer case of ambiguity in a patent application than one which would cause the patent owner in opposition proceeding to ask its experts to perform impossible experiments to support the alleged invention.

5.5 Dr. Aaronson says that he fails "to see the criticality of Dr. Alitalo's sequence data." (ASA2 at 27.) To me, it is plain that the data calls into question Dr. Power's credibility. One of the few aspects of her declaration that was independently verifiable was shown to be false, a fact which she now admits.

VI. DR. POWER'S DECLARATIONS ARE NOT OBJECTIVE AND HER EXPERIMENTAL DESIGN IS NOT BASED ON THE TEACHINGS OF THE OPPOSED APPLICATION.

6.1 Dr. Power's "shortcut" taken in her first declaration but not reported to the Patent Office was apparently based on the premise that the ATCC 97149 clone contained the sequence of the ATCC 75698 clone, but she apparently never performed the sequencing experiments that would have been needed to confirm this. This is an example of how Dr. Power's experiments were driven by a desired result, and not by objective science. This bias is aptly stated in ASP2 where she explains that her "understanding of the goals of the experiments described in Power Declaration I was to demonstrate that the 350 amino acid form of VEGF-2 could be successfully expressed and secreted . . . ." (See ASP2 at 7.) Dr. Power' second declaration again is defective because it is not based on the teachings of the opposed application. She is motivated by the question of whether she can achieve a result, rather than the question of whether the opposed application contained adequate teachings.

A. Lack Of Objectivity In Experimental Purpose And Design

6.2 For example, Dr. Power says that she has "now been asked to redesign my experimental protocol to specifically use the VEGF-2 coding sequence contained in ATCC Deposit No. 75698 to generate the expression construct containing the 350 amino acid form of VEGF-2." (ASP2 at 8.) I agree with the conclusions of Dr. Alitalo at OKA2 at 5.4, that this is impossible because HGS did not deposit a clone containing 350 amino acid

codons. (See OKA2 at 5.4.) One must depart from the teachings of the application, using hindsight, to do what Dr. Power has been asked to do.

6.3 Dr. Power says that "Even though ATCC Deposit No. 75698 lacks the complete coding sequence for the 350 amino acid form of VEGF2, a molecular biologist as of March 1994 would be able to recreate the 350 amino acid form of VEGF2 given the description of the complete sequence in the HGS patent specification . . . ." Dr. Power seems to be ignoring the guidance in the application that, to the extent the sequences in the application and the deposit differ, it is the deposit that is supposed to be "controlling." (See opposed application at p.9.)

6.4 Likewise, Dr. Power's stated purpose is "to determine whether the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence would result in the expression and secretion of the protein from eukaryotic cells." I agree with the conclusions of Dr. Alitalo at OKA2 at 5.3 that such experiments have no basis in the opposed application.

B. Irrelevant Results

6.6 In my opinion, Dr. Power's results are not conclusive in any event. The gels do not show "bands" at 30 kD. Rather they show smears of indeterminate size. One explanation for the smears is that she significantly overloaded the gels, which might also explain the very dark sections at the top of her gels.

6.7 Interestingly, Dr. Aaronson says that Dr. Power observed "a protein which resolves as a doublet at approximately 30 kDa. (See ASA2 at 28, citing ASP2 at 31.) Dr. Aaronson seems to be confusing his reading of the Alitalo file histories, which show a VEGF-C doublet, with his reading of Dr. Power's declaration, because Dr. Power characterized her results as indicating "a broad band resolving at approximately 30 kDa . . . ." (See ASP2 at 31.)

VII. DR. POWER'S RESULTS OWE NOTHING TO THE TEACHINGS IN THE APPLICATION.

7.1 Dr. Power's experiments were not a replication of any teachings in the patent application. Thus, irrespective of the results she obtained, they would be irrelevant.

7.2 Moreover, her results are again, irrelevant. Dr. Power reports for the second time that her experiments produced a polypeptide of approximately 30 kDa. (ASP2 at

31.) The opposed application makes no mention of the 30 kDa species of polypeptide observed by Dr. Power, or of a method of making it, or that one should expect to achieve it. This size simply is not described in the opposed application. The only "expression" experiment in the application (Example 2) produced a polypeptide of 38-40 kD. Dr. Power's second declaration sheds no more light on the lack of relevance of her data than her first one did, even though Dr. Alitalo explicitly raised the question in his second declaration. (See OKA2 at 5.6.)

7.3 HGS's declarants dismiss the molecular weight problem by declaring that neither they nor their colleagues would have required this information from the HGS patent application. (ANH2 at 1.9; ASA2 at 15.) Drs. Hayward and Aaronson have missed the point entirely. Dr. Alitalo was commenting on the relevance of the experiments by Dr. Power to the opposed application. Irrespective of whether patents are supposed to teach molecular weights, the HGS patent application does teach a molecular weight for VEGF2 (38-40 kD), as noted in the preceding paragraph. Dr. Power did not express a protein having that weight and this discrepancy is not explained. Dr. Power's results (in both declarations) have no apparent significance to the teachings of the opposed application.

7.4 Similarly, Dr. Aaronson implies that Susan Power's work demonstrates "the correct expression, secretion, and processing of the mature form of VEGF-2." (See ASA2 at 5, 7, 13-15.) In my opinion, neither Susan Power nor the other HGS declarants have provided any evidence that the substance produced by Dr. Power in either set of experiments is "correct" or corresponds to any form of VEGF2 purportedly taught in the opposed application. Dr. Alitalo has shown that the patent application's teachings regarding expression, secretion, and processing were incorrect.

VIII. THE CONTINUED ASSERTION THAT VEGF2 AND VEGF-C ARE THE SAME MOLECULE.

8.1 HGS continues to assert that VEGF2 and VEGF-C are the same molecule. (See, e.g., ASA2 at 3, 11.) Ludwig has already provided a detailed explanation as to why this assertion is deceptive in this proceeding, which I repeat here by reference. (See, e.g., OJB2 at 3.3-3.18; OPR2 at 2.13-2.16.) For example, VEGF-2 as defined in the opposed application is neither the complete prepro-VEGF-C sequence of 419 amino acids nor a properly processed form lacking, e.g., the large C-terminal propeptide. Notwithstanding

these and other differences, HGS for some reason has decided to introduce the United States VEGF-C prosecution histories of the Alitalo VEGF-C patents into this proceeding. The following comments are directed specifically to issues raised by these new documents germane to the issues of this proceeding.

A. HGS cites the Heldin declaration out of context.

8.2 Dr. Aaronson alleges that a declaration filed by Dr. Heldin during prosecution of the Alitalo application and pertaining to issues of inherent properties of a protein has some relevance to this proceeding. (ASA2 at 16-17, 20.) However, it is clear from the entirety of that declaration that Dr. Heldin is talking about research actually performed by Dr. Alitalo's group, and not the subject matter of the opposed application. There are no issues of inherency and polypeptide properties with the HGS application because there was no polypeptide research reported in the HGS application. HGS filed its patent application after sequencing what was later admitted to be an incomplete cDNA, and after performing one "in vitro" expression experiment. (Example 2 of the opposed application.) HGS explicitly taught what they considered to be the amino acid sequence of their "full length" (~350 amino acid) and "mature" forms (~326 amino acids) of VEGF2 and explicitly taught a molecular weight (38-40 kd) for their expression product. It is irrelevant and misleading for HGS to refer to issues of inherent properties of proteins because the opposed application already teaches these properties as they were understood by HGS and as HGS wished to convey them to the public.

8.3 The Aaronson declaration casually makes reference to "the 30 kDa doublet and 23kDa secreted forms of VEGF-2" and alleges that Dr. Alitalo's work (embodied in his own patent application files) shows expression of VEGF2 in a variety of cell types. (ASA2 at 17, 21-22, 30.) Insofar as they are suggesting that Dr. Alitalo has expressed and secreted VEGF2 *as taught in the opposed application*, they are grossly misleading the Australian Patent Office. Dr. Alitalo's work reported to the U.S. PTO involved his VEGF-C molecules, which were neither disclosed nor suggested by the opposed application. Dr. Alitalo's work based on the teachings of the opposed application is embodied in the declarations that he has filed in this proceeding. Collectively, all of these documents demonstrate that Dr. Alitalo is fully capable of performing competent expression experiments, and that VEGF2 as taught in the opposed application cannot be expressed and secreted because it is incomplete. It appears to me that HGS is attempting to confuse the Australian Patent Office and take credit for Dr. Alitalo's work.

8.4 The Australian Patent Office should find the inclusion of the Alitalo patent files enlightening because of their rich and detailed discussion of protein expression, secretion, and mutation studies. They stand in sharp contrast to the opposed application, which provides an incomplete cDNA sequence, a defective Northern hybridization study, a meaningless and inadequately described "in vitro" expression experiment, and little else. [OPR1 at 4.13-4.13.3; OJB2 at 5.1-5.5.] The differences in the amounts of true scientific information provided to the reader are striking. I find it troubling that HGS would try to take credit for research and discoveries by the Alitalo research group that owe nothing to the teachings of the opposed application.

B. The prosecution of the Alitalo patents confirms that Alitalo's VEGF-C work is a patentably distinct invention from the HGS VEGF2 work.

8.5 If the Australian Patent Office needs further explanation distinguishing VEGF2 of the opposed patent from Dr. Alitalo's VEGF-C work, it need look no further than the Alitalo prosecution documents introduced into evidence by HGS. The salient points that one can observe from these file histories include the following:

8.6 The Alitalo group submitted various HGS documents to the U.S. Patent Office as part of "information disclosure statements." Ludwig Institute's attorneys have explained to me that an information disclosure statement is a vehicle to ask an examiner to consider certain literature when examining the merits of a patent application. The documents that were sent to the U.S. Patent Office for consideration include WO 95/24473, which is the original published version of opposed application, and also related HGS U.S. patent applications 08/270,550, filed 08 March 1994, 08/465,968, fled 06 June 1995, and U.S. Patent Nos. 5,932,540 and 5,935,820.

8.7 To secure allowance of their U.S. Patents, the Alitalo group explained to the U.S. Patent Examiner that the correctly processed VEGF-C forms that were the subject of their claims were neither disclosed nor suggested by HGS in its VEGF2 applications. The U.S. Patent Office agreed, and allowed the Alitalo patents to issue.

IX. UNCONTESTED ASPECTS OF LUDWIG'S EVIDENCE IN REPLY

9.1 None of the HGS Declarations respond directly to any issues raised in the second Rogers and Ballard declarations. Given that HGS was granted an opportunity to

submit further evidence, it is interesting to observe just a few of the issues that HGS has left uncontested.

9.2 HGS has failed to directly contest the detailed explanation of why the terms "VEGF-C" and "VEGF2" in the context of this opposition proceeding mean very different things, and that it is deceptive to use them interchangeably. (See, e.g., OPR2 at 2.13-2.16; OJB2 at 3.3-3.18)

9.3 HGS has failed to contest specific allegations that their declarants rely, inappropriately, on hindsight to misstate the amount of experimentation required to make the invention work. (See, e.g., OPR2 at 4.54-4.71; OJB2 at 8.1-8.11)

9.4 HGS failed to contest detailed explanations as to why terms such as "VEGF2 activity"; "fragment, analog or derivative"; and "hybridization" are indefinite with respect to the claims of the opposed patent. (See, e.g., OPR2 at 3.3-3.23.3; OJB2 at 12.2-12.14)

9.5 HGS fails to dispute that its declarations in answer applied a scientific double standard when analyzing scientific data. (See, e.g., OPR2 at 2.2-2.7 and 2.9-2.10)

9.6 HGS fails to contest that VEGF2 is too distantly related to prior art molecules to have fairly predicted the structure and function of VEGF2. (See, e.g., OPR2 at 4.7-4.11.)

X. CONCLUDING REMARKS

10.1 Having examined the opposed application and all of the declarations that have been submitted by both parties, the following conclusions are self evident: (1) HGS asked Dr. Power to perform lots of experiments related to the VEGF2 subject matter, but carefully avoided asking her to test (or report) the direct teachings in the application, i.e., whether VEGF2 of about 350 amino acids is expressed and secreted by cells. (2) Dr. Alitalo's experiments tested this question and showed, multiple times, that expression and secretion does not occur. (3) HGS asked other scientists to criticize Dr. Alitalo's work, but not actually test it themselves (or report the results of such tests). As a result, the only experimental evidence testing the teachings of the opposed application is the evidence reported by Dr. Alitalo.

AND I MAKE this solemn declaration by virtue of the Statutory Declarations Act 1959, and subject to the penalties provided by that Act for the making of false statements in statutory declarations, conscientiously believing the statements contained in this declaration to be true in every particular.

DECLARED at ...ADELAIDE.....

This 16th day of July, 2002

Before me:

(Signature of Witness)

Anne Hinton ACA
Chartered Accountant

Francis John Ballard

AUSTRALIA

Patents Act 1990

IN THE MATTER OF Australian Patent Application Serial No. 696764 by Human Genome Sciences, Inc.

-and-

IN THE MATTER OF Opposition thereto by Ludwig Institute for Cancer Research

STATUTORY DECLARATION

I, Kari Alitalo, a Research Professor of the Finnish Academy of Sciences, at The Molecular/Cancer Biology Laboratory, Biomedicum Helsinki, P.O.B. 63 (Haartmaninkatu 8) 00014 University of Helsinki, Finland, do solemnly and sincerely declare as follows:

I. INTRODUCTION

1.1 In February, 2000, I executed a first statutory declaration (hereinafter referred to as "OKA1" (Opponents, Kari Alitalo, 1st Declaration)) to provide experimental evidence in support of the opposition filed by Ludwig Institute for Cancer Research ("Ludwig Institute") to the issuance of a patent to Human Genome Sciences, Inc., ("HGS") based on HGS's Australian Patent Application No. 696764 ("the opposed application"). That first declaration included a brief summary of my scientific credentials and an introduction in which I set forth some conventional terminology and relevant background information regarding VEGF-C and signal peptides. The first declaration summarized controlled experiments which demonstrated that VEGF2 as taught in the opposed application cannot be expressed and secreted by cells.

1.2 I executed a second statutory declaration (hereinafter "OKA2") in September, 2001. My OKA2 declaration responded to criticisms of the experiments in OKA1

alleged in declarations by Jennifer Ruth Gamble (hereinafter "AJG1"), Nicholas Kim Hayward (hereinafter "ANH1"), and Stuart A. Aaronson (hereinafter "ASA1"), filed on behalf of HGS (OKA2 at 1.2). I repeated and revised the experiments reported in OKA1 in order to address every criticism alleged by HGS in the first group of HGS declarations. The data again showed that cells cannot express and secrete VEGF2 as taught in the opposed application.

1.3 HGS then filed second declarations by Dr. Hayward (hereinafter "ANH2") and Dr. Aaronson (hereinafter "ASA2") critiquing the experiments performed in OKA2. Ludwig Institute asked me to design and perform further protein expression experiments that would address any criticisms raised in ANH2 and ASA2. Further, Ludwig Institute asked me to comment on the data from expression studies performed by Dr. Susan Power and reported in her second declaration (hereinafter "ASP2"), filed at the same time as ANH2 and ASA2.

1.4 I hereby reaffirm my understanding that I have an overriding duty to the Patent Office (and to any Australian Federal Court that should review the Patent Office decision) to provide objective scientific analysis that I believe to be truthful. I hereby affirm that, to the best of my knowledge and belief, factual statements herein are true and opinion statements herein represent my objective scientific opinion and analysis.

II. EXPERIMENTS TO ADDRESS CRITICISMS REGARDING ALLEGED LACK OF CONTROLS, TRANSFECTION EFFICIENCY AND ANALYSIS OF EXPRESSION AND SECRETION OVER MORE THAN ONE TIME POINT

2.1 At the outset, I observe that HGS declarants failed to raise any of these criticisms when commenting on my first declaration. Had they done so, I would have addressed and reported these details in my second declaration. The transfection efficiency and time course experiments relate to rather conventional procedural details, and I am not accustomed to being asked to report them (e.g., when I submit manuscripts of my research to journals for publication).[1] The criticisms alleged in the latest HGS declarations do not cause me to change

[1] The HGS declarants continue to argue that the opposed application teaches to construct expression vectors in which the 350 amino acid VEGF2 sequence is fused in frame with a (heterologous) signal sequence. (See, e.g., ASA2 at 6-8.) I have already explained that my experimental design is based on actual teachings of the opposed application, whereas the experiments now suggested by HGS are not. (See, e.g., OKA at 2.2-2.3 and 5.3.)

my conclusions expressed in my earlier declarations, and I still believe that all of the data from those declarations is sound.

2.2 The following analysis provides evidence that the data provided in OKA1 and OKA2 is accurate and credible, and further supports the position that cells cannot express and secrete VEGF2 as taught in the opposed application.

A. Experimental procedure

1. Cells and Plasmids:

3.1 Results reported in OKA2 revealed that COS and 293T cells were equally appropriate cell lines for analyzing VEGF2 protein expression and secretion. For these new experiments, 293T cells were grown in DMEM supplemented with 10 % fetal bovine serum, glutamine and penicillin/streptomycin.

3.2 The polymerase chain reaction (PCR) was employed to construct a cDNA fragment that corresponded to amino acids 70 to 419 of prepro-VEGF-C. For the purpose of these experiments (directed to assessing transfection efficiency and protein expression at various time points) the cDNA fragment encoding amino acid residues 70 to 419 of prepro-VEGF-C corresponds appropriately with the cDNA encoding the full length sequence of the VEGF2 polypeptide described in the opposed application. Nucleotides 559 to 1608 of the VEGF-C cDNA (Reported in Document D70, Joukov et. al. 1996, GenBank accession number X94216) were PCR amplified with the primers 5′-CGCGGATCCATGACTGTACTCTACCCA-3′ containing a BamHI site and 5′-CGCTCTAGATCAAGCGTAGTCTGGGACGTCGTATGG-GTACTCGAGGCTCATTTGTGGTCT-3′ containing a XhoI site, HA-tag, a stop codon and a XbaI site and cloned into pcDNA1(Amp)-vector (Invitrogen). The resultant vector was designated as VEGF-2(HGS)/pcDNA1.

3.3 As previously reported in OKA2, an expression vector was also constructed that contained the full length (419 codons) VEGF-C sequence (OKA2 at 3.3.3) for use as a positive control in the expression and secretion analyses. The resultant vector was designated as VEGF-C/pcDNA1.

2. Transfection and time course:

3.4 A principle criticism alleged by the HGS experts was that my OKA2 declaration failed to include transfection efficiency data (ANH2 at 1.5; ASA2 at 24). Thus, for these new experiments, two separate expression vectors, pRL expressing Renilla Luciferase (Promega) and pCMV-ß-gal expressing ß -galactosidase under CMV promoter, were used as transfection controls.

3.5 The other principle criticism of the procedures reported in my OKA2 declaration was regarding the lack of time points in the expression analyses (ANH2 at 1.5; ASA2 at 25). To address this concern three different time points were tested in the new experiments. In particular, the 293T cells were split 1:6 and fresh medium was changed 19 hours thereafter. Three hours after medium change, VEGF-2(HGS)/pcDNA1, VEGF-C/pcDNA1, or empty vector were co-transfected with either pRL (three plates with each combination in a 16:1 ratio), or pCMV-ß-gal (one plate with each combination in a 8:1 ratio), using FuGENE6 Transfection Reagent (Roche). The conditioned media and the cells were harvested 24 hours, 48 hours, or 72 hours after the transfection for the purpose of evaluating protein expression and secretion at these different time points. Either twenty-four hours (for time points 48h and 72h) or sixteen hours (for time point 24h) prior to harvesting, the cells were washed twice with PBS and changed to 3 ml of MEM medium containing 100 µCi/ml ^{35}S-methionine and ^{35}S-cysteine (Promix, Amersham) for metabolic labeling of proteins synthesized by the cells[2]. At the indicated time points the conditioned media was harvested and cleared by centrifugation. The cells were trypsinized, washed twice with PBS and lysed in 1X passive lysis buffer from the Dual-Luciferase Reporter Assay System (Promega).

3. Immunoprecipitation:

3.6 Immunoprecipitation experiments were conducted to identify the presence of the various VEGF-C or VEGF2 polypeptides in the conditioned media.

3.7 For immunoprecipitation, 1.25 ml of each conditioned media was supplemented with BSA and Tween 20 to final concentrations of 0.5% and 0.02%, respectively. The different VEGF-C peptides were immunoprecipitated with polyclonal antibodies raised

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[2] See explanation in OKA2 at 3.4.2

against a synthetic peptide corresponding to amino acid residues 104-120 of the VEGF-C prepropeptide (Antisera 882, reported in Document D71, Joukov et al., 1997) at 4 °C for 2 hours. This peptide is present in the secreted form of VEGF-C, and the opposed application teaches that it should be present in mature VEGF2 as well. Thus, antisera raised against this peptide should recognize VEGF2 or VEGF-C polypeptides produced by the cells.

3.8 The immunocomplexes were precipitated with protein A-Sepharose for 1 hour and washed 2 times with 1X binding buffer (0.5% BSA, 0.02% Tween20 in PBS) and once with PBS at 4°C. The proteins were analyzed by SDS-PAGE in a 12 % gel under reducing conditions. Half (50%) of each immunoprecipitate sample was loaded into each lane. Since cell cultures contained equivalent amounts of media and equal volumes were used for immunoprecipitation and loading, each lane received an equal sample aliquot for analysis.

4. Luciferase assay:

3.9 The protein concentrations of the cell lysates were determined by the BCA Protein Assay (Pierce) and the luciferase activity in the lysates was measured with the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions.

5. ß-galactosidase staining:

3.10 The cells were washed twice with PBS, fixed with 0.05% glutaraldehyde in PBS for 15 minutes at room temperature, washed three times with PBS, and stained over night with 2.5 mg/ml X-Gal (5-bromo-4-cloro-3-indolyl-ß-D-galactopyranoside) in 5 mM $C_6FeK_4N_6$ (kaliumferrocyanid) and 5 mM $C_6FeK_3N_6$ (kaliumferricyanid), 2 mM MgCl2, 0.01 % sodium deoxycholate, 0.02 % Nonidet P-40 in 0.1 M phosphate buffer, pH 7.3 at 37°C.

B. Experimental results

4.1 VEGF-2(HGS)/pcDNA1, VEGF-C/pcDNA1, or the mock vector were co-transfected into 293T cells with a plasmid encoding the Renilla luciferase gene and the secretion of VEGF-C into the conditioned medium as well as the luciferase activity in the cells were analysed 24, 48, and 72 hours after transfection.

4.2 Similar to the results reported in QKA2, no VEGF2 protein was detected in the conditioned media from the cells transfected with the VEGF-2(HGS)/pcDNA1 construct at

any of the time points tested, over a time period of 72 hours (Exhibit KA-1 Figure, Lanes 1, 4 and 7). In contrast, VEGF-C protein was effectively expressed and secreted by cells transfected with a vector encoding the full length VEGF-C. The different forms of VEGF-C immunoprecipitated from the conditioned medium correspond to partially and fully processed forms of VEGF-C (Exhibit KA-1 Figure, Lanes 2, 5 and 8). These results are in agreement with the results reported in OKA1 and OKA2, and provide evidence that VEGF2 as taught in the opposed application cannot be expressed and secreted by cells.

4.3 To address criticisms suggesting low expression levels of VEGF2 were a result of poor transfection, transfection efficiency was tested in two ways. In one experiment, luciferase activity of the cell lysates was measured and the relative light units/µg of protein were found to be comparable in the cells transfected with VEGF-2(HGS)/pcDNA1 and VEGF-C/pcDNA1. Because the co-transfected luciferase plasmid encodes an enzyme that causes production of light waves under the assay conditions that were used, the measurements of light units provides an indication of the relative transfection efficiency of the cells. In a second experiment, transfection efficiency was also analysed by ß-galactosidase staining of separate plates in which the vectors were co-transfected with a plasmid coding for ß-galactosidase. The ß-galactosidase is an enzyme that causes production of a colorometric product, under the assay conditions used, and thus produces an independent measure of relative transfection efficiency. Results of the transfection efficiency analysis using either of these controls revealed that the vectors were introduced into cells equivalently in the transfections, and thus ruled out the possibility of poor transfection as a cause for the absence or decreased level of VEGF2 protein expression.

4.4 To address criticisms suggesting that the absence of VEGF2 expression was attributable to the length of time that the cells were cultured in the OKA2 experiments, cells were cultured in these experiments for various lengths of time prior to harvesting the cells or media. As the figure shows, cells do not express and secrete VEGF2, no matter what time point is used to terminate the experiment (see Exhibit KA-1). Expression of VEGF-C polypeptides, which serves as a positive control, was visible at all time points studied. Expression was already visible at 24 hours and was strongly visible at 48 hours and 72 hours.

C. Conclusions

4.5 My experiments reported in OKA2 were intended by me to respond to the criticisms raised by HGS in its first series of declarations. Drs. Hayward and Aaronson attempted to discredit the experiments in OKA2 by pointing to alleged new flaws in the experimental design that they failed to raise in their first declarations (ANH2 at 1.5; ASA2 at 5). As reported above, I have now conducted another set of experiments that directly addresses the new criticisms raised in ANH2 and ASA2. The result of those experiments has, once again, revealed that cells cannot express and secrete a VEGF-2 protein as taught in the opposed application.

III. COMMENTS ON EXPERIMENTS PERFORMED BY DR. POWER IN ASP2

A. Comments regarding Dr. Power's experimental Procedures

5.1 In my second declaration I explained that Susan Power's experimental design and results have nothing to do with the teachings in the opposed application. I remain of that opinion. (See. e.g., OKA2 at 2.2-2.3 and 5.3.) The same analysis is true of her second set of experiments reported in ASP2, and I repeat that analysis by reference.

5.2 In my second declaration I explained that Susan Power could not have used the starting materials (ATCC Clone 75698, referred to in the opposed application as amended) that she said that she used in her experiments. (See OKA2 at 5.3.) She has confirmed that my analysis of this issue was correct. (See ASP2 at 5-6.)

B. Comments regarding Dr. Power's experimental results

5.3 In my second declaration I observed that the approximately 30 kDa protein reported in the Power experiments had seemingly no relevance to the opposed application, which makes no mention of this species of polypeptide. (See OKA2 at 5.6.) No explanation was given by Dr. Power in her second declaration to explain the significance of this protein to the opposed application, even though, in her second set of experiments, she again reports "a broad band resolving at approximately 30 kDa." (ASP2 at 31.) In my opinion, to the extent the opposed application teaches anything about protein size, it teaches the 350 amino acid VEGF2, the mature VEGF2 of about 326 amino acids (application at p. 5), and *in vitro*

expression of proteins of 36-38 kD or 38-40 kD (Example 2). There is no mention or suggestion of a 30 kDa band.

5.4 The gels embodying Dr. Power's results deserve one additional comment.

5.5 Although Dr. Power did not describe in detail the volumes in her sample loading in her second declaration, it appears to me that she loaded larger (or more heavily concentrated) samples from some transfection experiments than others. This has had the effect of making the results of interest (the bright colored bands) in different lanes and different gels look identical to each other. The evidence supporting this conclusion is the presence of very black spots that appear between the 50 and 64 kD lane markers in the samples derived from cell supernatant (medium). 5.6 Dr. Power does not explain what these dark spots are, or even acknowledge their existence. In my opinion, since the black spots only appear in the even-numbered "supernatant" lanes, it would appear that the spots reflect a proteinaceous component of the growth medium that Dr. Power was using and that would have been transferred to the gels in the "supernatant" lanes. The likely candidate would be serum albumin proteins that were present in the growth medium used by Dr. Power. (See ASP2. at 22. Dr. Power concentrated the medium (and thus the proteins in it) before running it on her gels. ASP2 at 23.)

5.7 Had Dr. Power loaded comparable amounts of sample in each lane, and in each gel, then I would have expected comparable amounts of serum albumen in each lane, and hence, similar sized black spots (or no spots whatsoever) in all supernatant lanes. However, the black spots have different intensities, suggesting to me that Dr. Power may have loaded different amounts of samples to affect the way that her bright bands appear on the gel. For example, some of Dr. Power's "24 hour" lanes have more prominent dark spots than her 48 hour lanes. (Compare spots in Gel 3, lanes 2, 4, or 6 to Gel 3, lane 8.) Also, the dark spots in Gel 1 appear darker than Gel 2 or 4.) Thus, Dr. Power appears to have been adjusting the size of her samples, so that her "bright spots" would appear the same in her various experimental samples.

IV. CONCLUDING REMARKS

6.1 The protein expression and secretion studies I report herein were designed to address any criticisms made by HGS with regard to experimental design credibility. The

results demonstrate several key points. First, VEGF2 as taught in the opposed application cannot be expressed and secreted by cells. The data clearly establishes the failure of VEGF2 to be expressed and secreted at multiple time points over a period of 72 hours (Each of these time points was sufficient to observe expression and secretion of the VEGF-C positive control run under the same experimental conditions). Second, transfection experiments reported herein rule out the possibility that poor expression of VEGF2 was due to lower amounts of the VEGF2 expression vector being introduced into the cells. Thus, controls, transfection efficiency and expression and secretion over time have all been accounted for in this declaration.

AND I MAKE this solemn declaration by virtue of the Statutory Declarations Act 1959, and subject to the penalties provided by that Act for the making of false statements in statutory declarations, conscientiously believing the statements contained in this declaration to be true in every particular.

DECLARED at HELSINKI FINLAND

This 16 day of July, 2002

Kari Alitalo

BEFORE ME:

(Signature of Notary Public)

LAURI HAIKARAINEN
Notary Public

HELSINGIN MAISTRAATTI
MAGISTRATEN I HELSINGFORS

SPRUSON & FERGUSON

AUSTRALIA

Patents Act 1990

IN THE MATTER OF Australian Patent Application Serial No. 696764 by Human Genome Sciences, Inc.

-and-

IN THE MATTER OF Opposition thereto by Ludwig Institute for Cancer Research

THIS IS Exhibit KA-1

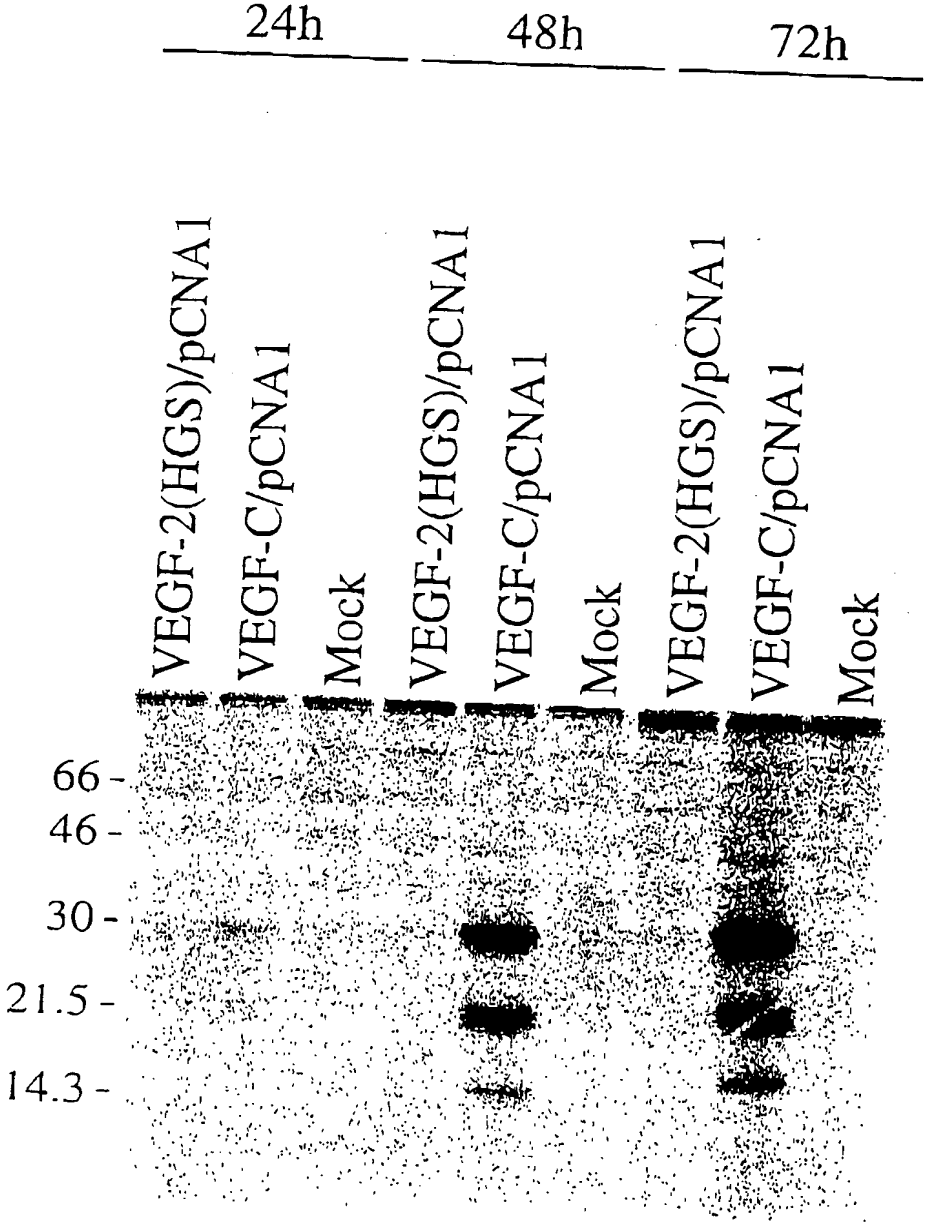
referred to in the Statutory Declaration

of Kari Alitalo

made before me

DATED this Day of July 2002

(Signature of Notary Public)


24h
48h
72h
VEGF-2(HGS)/pCNA1
VEGF-C/pCNA1
Mock
VEGF-2(HGS)/pCNA1
VEGF-C/pCNA1
Mock
VEGF-2(HGS)/pCNA1
VEGF-C/pCNA1
Mock
66 -
46 -
30 -
21.5 -
14.3 -

Figure